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SOME IMPLICATIONS OF AUTOTROPHIC SULFUR AND IRON BACTERIA IN WATER POLLUTION BY URANIUM MILL TAILINGS WASTES IN THE ELLIOT LAKE AREA.

by

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- Introduction -

The water quality of a considerable portion of the Serpent River watershed in the Elliot Lake region has been adversely affected by the acid and mineral pollution resulting from the waste tailings discharge by uranium mining and milling operations.

Oxidation of metallic sulfide, chiefly pyrite (FeS_2) in tailings wastes exposed to atmosphere has resulted in the formation of sulfuric acid and soluble iron which have entered streams and lakes through seepage and run-off from the tailings impoundments. Detrimental effects on the biota of these waters have been caused by the depressed pH condition, and increased or abnormally high concentrations of sulphate, metallic cations and radionuclides.

It is well known that the activities of microorganisms have played a role in the formation and destruction of rocks, oil and sulfur deposits and are significant in other geological transformations in nature.

The purpose of this investigation was to determine some implications of the autotrophic sulfur and iron oxidizing bacteria in the acidification of surface waters by the uranium tailings wastes.

CHARACTERISTICS OF IRON AND SULFUR BACTERIA

The autotrophic sulfur-oxidizing bacteria of the genus Thiobacillus are ubiquitous in soil and water as natural agents of the sulfur cycle. Certain species of this genus are typically found in such habitats as sulfur springs, acid drainage waters from coal mines and deposits of sulfide ores exposed to air.

These pseudomonads are aerobic, chemosynthetic autotrophs which obtain their energy for growth from the oxidation of reduced inorganic sulfur compounds, and in a particular case from the oxidation of reduced iron.

As autotrophs, the bacteria grow on inorganic media, using CO_2 as their sole source of carbon, and ammonia as the preferential source of nitrogen.

Although some workers have reported that growth of the thiobacilli is inhibited by organic matter (1, 23, 47), recently it has been shown that some strains of the thiobacteria are facultative autotrophs and may utilize certain organic compounds for heterotrophic growth as well (7, 37, 43).

Thiobacillus thiooxidans shows optimum growth at pH 2 - 4 and fails to grow above pH 6 (42). This organism thus oxidizes sulfide, elemental sulfur and thiosulfate in acid habitats, but does not metabolize inorganic iron compounds (33).

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Thiobacillus ferrooxidans is also an acidophilic autotroph which can metabolize sulfur, thiosulfate, insoluble metallic sulfides but in addition, ferrous iron [21, 22, 44]. Growth of this microorganism usually ceases when the pH exceeds 4.5 [23]. Tolerance of T. ferrooxidans to increasing concentrations of copper and some other metallic cations has been reported (4, 34). The acidophilic thiobacteria can withstand fairly large concentrations of H_2SO_4.

Ferrobacillus ferrooxidans, first reported by Leathen et al. [26], is closely related to T. ferrooxidans, except that it can use only Fe^{+2} as an energy source and is unable to utilize reduced sulfur compounds for its autotrophic existence. In fact, the validity of the species Thiobacillus ferrooxidans was questioned by Leathen et al. (24, 25) who failed to find a single bacterium capable of oxidizing both thiosulfate and ferrous iron. These authors believed that the acidification of bituminous coal mine effluents could be ascribed to two bacteria, Thiobacillus thiooxidans and Ferrobacillus ferrooxidans, the former oxidizing sulfur to sulfuric acid, the latter oxidizing ferrous iron as its sole oxidizable substrate.

However, Bryner & Jameson (5) and Razzell and Trussell (34) named the strains they isolated from acidic ore leach waters, Thiobacillus ferrooxidans because the isolates oxidized ferrous iron in addition to sulfur and metallic sulfides.

Margalith et al. (28) reported on the growth kinetics of Ferrobacillus ferrooxidans on both iron and sulfur substrates. Sulfur-grown cells oxidized Fe^{+2} at a rate similar to that of iron-grown cells, which indicated that the iron oxidation system of F. ferrooxidans is constitutive. The sulfur oxidation system was inducible, as evidenced by a lag in sulfur oxidation when iron-grown cells were transferred to sulfur media or no lag when sulfur-grown cells were transferred to sulfur media.

ROLE OF IRON AND SULFUR BACTERIA IN THE OXIDATION OF SULFIDE ORES IN NATURE

In nature, T. ferrooxidans is active wherever sulfides are exposed to the earth's surface or where oxygen-rich waters come in contact with sulfides (23). Pyritic minerals are normally chemically stable in the alkaline and reducing environment of the undisturbed strata, but when exposed to the atmosphere they oxidize under the influence of biological and/or non-biological forces to form water soluble acid sulphates. Pyrite (FeS_2) is chiefly the source of acid, iron and sulfate in mine drainage (15).

The acidophilic sulfur and iron autotrophs of the Thiobacillus - Ferrobacillus group have been commonly isolated from the acid drainage water of coal mines, etc. Clark (9) estimated that bacteria were responsible for approximately 80% of the acid formed in some

coal mines. Microbiological activity is involved in the oxidation of pyrites and atmospheric oxygen is not the sole factor in the formation of acid mine drainage (5, 10).

Moreover, bacterial leaching by these autotrophic micro-organisms has been used successfully for the commercial production of uranium (17, 27, 31). Various metals, e.g. Mo, Cu, Co, Ni, have been recovered from low grade ores by microbiological oxidation of the metallic sulfides (4, 13).

POTENTIAL CONTROL MEASURES FOR PREVENTION OF ACID FORMATION IN MINE WASTES

Reports on the pollution of surface waters (20, 22) and ground water (15) by the acid drainage from coal mines and discussions of mine drainage problems (2, 32) can be found in the literature.

Several techniques for discouraging or preventing the generation of acid in mines have been proposed. These include hydrologic isolation of mines, mine sealing, passivation, use of bactericidal agents and reduction of sulphates (2). Barnes and Romberger (2) have considered the less desirable but often effective alternative of neutralizing the acid water with limestone.

Glover (20) tested a process for the treatment and purification of acid mine drainage on a pilot plant scale. Basically, the process involved the biochemical oxidation of ferrous salts in acid solution and subsequent neutralization of the acid by pulverized

limestone. Since complete oxidation of all iron was necessary for stabilization of pH, the process was only applicable for more dilute acid drainages.

Although no bacteriophage has ever been isolated which attacks Thiobacillus spp., there is some evidence for the existence of a viable antibacterial agent, presumably a virus specific for Thiobacillus - Ferrobacillus bacteria (16, 38). Potentially, phage inoculation might be useful in the control of the acid producing bacteria in acid mine waters.

Control of bacterial growth by chemical bactericides has been suggested. Sodium azide and N-ethylmaleimide selectively inhibit iron and sulfur oxidation respectively (14), and quaternary ammonium compounds appear to be quite effective in inhibiting respiration of F. ferrooxidans (8). Schnaitman et al. (36) have suggested that two good inhibitors of iron oxidation, formate and molybdate, may potentially be of some importance in controlling water pollution from acid mine wastes.

However, most attempts at using bactericidal agents to control these bacteria under natural conditions have been unsuccessful. Treatment of the entire system is usually impossible because of complications in local hydrology and besides, these bacteria can recover rapidly from attempts at poisoning and re-inoculate themselves in mines (2). Many of the organic bactericides are degraded and lose their effectiveness rapidly, while toxic inorganic compounds,

e.g. cyanide, azide, arsenates produce undesirable side effects and toxicities towards other forms of life in the affected waters.

Tuttle et al. '46 suggested an acid mine drainage abatement procedure by the use of microbial sulfate reduction. This method however appears to be only applicable to flooded mines where other techniques are also feasible.

I. DISTRIBUTION OF AUTOTROPHIC SULFUR AND IRON BACTERIA AT SELECTED SITES (surface waters and tailing areas) IN THE ELLIOT LAKE REGION

Reports dealing with the ecology of streams polluted by acid mine wastes have indicated that acid conditions could cause the death of the normal water microflora and that aciduric species, notably fungi could thrive (29, 45, 48, 49).

Tuttle et al. (45) found that normal non-acid streams contained relatively low numbers of acid-tolerant heterotrophic microorganisms. The acid-tolerant aerobes survived when acid entered the stream and actually increased in number under acid conditions. The predominating heterotrophs found in the acid waters were yeasts, molds and some gram negative Pseudomonas and Achromobacter (Acinetobacter) types. A Flavobacterium sp. isolated from a stream was inhibited by acidity (29). Gram positive aerobic and anaerobic bacteria (Bacillus and Clostridium) died out very rapidly in acidic water, but autotrophic iron and sulfur bacteria were present wherever mine water entered a stream system.

The autotrophic bacteria of the Thiobacillus - Ferrobacillus group are responsible for the enzymatic oxidation of ferrous iron and reduced inorganic sulfur compounds with the concomitant production of sulphate, ferric, and hydrogen ions. McCoy and Dugan (29) presented convincing evidence that a large proportion of the acidity, sulphate and metal ions in mine drainage are present as a result of

PROCEDURE

An ecological investigation of the chemoautotrophic sulfur and iron oxidizing bacteria was carried out in the Elliot Lake region from October 1968 to September 1969. The population distribution of these bacteria was determined at selected stations in surface waters and tailings areas affected by uranium mining and milling operations.

Sampling stations were selected at the following mill locations: Rio Algom (Nordic), Stanrock and Denison Mines Ltd. Surface water samples were taken in the tailings areas and in streams and lakes affected by run-off from the tailings areas in October 1968, July 1969 and September 1969. Depth samples of tailings waste were taken from a profile at the Nordic site during the summer of 1969. Water and tailings waste depth samples were analyzed for autotrophic sulfur and iron oxidizing bacteria of the Thiobacillus-Ferrobacillus group. In some cases, samples were also analyzed for sulphate reducing bacteria.

A most probable number (MPN) method was employed for the enumeration of the autotrophic bacteria in the samples. Broth media used for the cultivation of the sulfur and iron oxidizers were basically those described by Postgate (33) for Thiobacillus thiooxidans and Ferrobacillus ferrooxidans. Sodium thiosulfate (5 g/l) was the energy source in the T. thiooxidans broth. The initial pH of this

medium was adjusted to 4.5 - 5.0, dispensed into test tubes and autoclaved at 15 p.s.i. for 12 min.

Subsequent investigation indicated that a modification of th F. ferrooxidans broth (33) resulted in improved growth of the iro bacteria. More rapid growth and better recovery of the iron oxidizers was found when the 9K medium of Silverman and Lundgren was used with the energy source, $FeSO_4$ increased to a concentratio of 4 g/l. Trace elements (33) were also added to both media to attain optimum nutritional conditions. The F. ferrooxidans broth prepared by dissolving $FeSO_4$ and all ingredients in distilled wate adjusting the pH to 4.0 - 4.5, dispensing into test tubes and auto claving at 15 p.s.i. for 12 min. It was found that the pH of the ferrooxidans broth dropped approx. 1 pH unit during autoclaving to the desired final pH of 3.0. No oxidation of iron occurred during autoclaving in the acid environment of the medium.

For the MPN procedure, a 3 tube series of each medium was inoculated with aliquots of the sample or dilution thereof. All tubes were incubated aerobically for 4 weeks at room temperature (approx. 25°C) in the dark.

All MPN tubes were checked for evidence of growth after incuba- tion. Determination of growth of the sulfur oxidizers was made by means of a pH spot test and a comparative sulphate test. Bacterial oxidation of thiosulfate produces sulfuric acid as the final metabol by-product.

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Although sterile tubes of thiooxidans broth remained clear after incubation, some inoculated tubes showed a cream-colored precipitation of elemental sulfur on the bottom and sides of the tube or sometimes the sulfur appeared as a fine pellicle on the surface of the broth.

A quick test for acidity and sulfate was made on each test tube of T. thiooxidans broth. A drop of broth from each tube in the MPN series was placed on a small segment of pH indicator paper (pH range 2 - 10). If the pH of the test medium had dropped appreciably from that of the sterile control tubes, a positive reading was designated for acid production.

An approximate determination for sulphate production in each test tube was made by adding 1 ml. of the test broth to a large test tube containing 18 ml. of distilled water. One ml. of a 2% $BaCl_2$ solution was then added to each large test tube and the contents shaken. A control tube containing 1 ml. of sterile thiooxidans broth was treated in the same way. Turbidity in each test tube after $BaCl_2$ addition was measured by visually comparing each tube with the control tube. Only a faint haze developed when $BaCl_2$ was added to the control.

A definite milky turbidity appeared in some tubes after addition of $BaCl_2$ solution. For the comparative visual turbidity test, the tubes were set in a test tube rack and viewed from the side and from above through the liquid column in the tube. Tubes were recorded as being positive for sulphate production from thiosulphate if $BaSO_4$

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turbidity appeared discernibly greater than the control. It was found that the sulphate concentration in media where growth of the sulfur oxidizers had occurred was several times greater than the sulphate concentration in sterile broth.

Growth of the autotrophic-iron oxidizing bacteria Ferrobacillus ferrooxidans or Thiobacillus ferrooxidans was determined after 4 - 6 weeks incubation by examining each test tube of culture medium for evidence of ferrous iron oxidation. No oxidation of iron occurred in sterile control tubes. A deep reddish-brown coloration with precipitation of ferric hydroxide on the bottom and walls of the test tubes developed in ferrooxidans broth giving a strong positive reaction. A more pale straw-yellow hue developed in tubes showing a slight positive reaction. In tubes giving a negative test, i.e. no growth of iron oxidizers, no change over the original pale green color of the uninoculated broth was observed.

Enumeration of the bacteria of each type was made by recording the number of positive determinations in each test for the MPN series and recording the count from a standard MPN table. Counts were related to number per 100 ml. for water samples or number of bacteria per gram air dried tailings waste.

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TABLE I - Populations of autotrophic iron and sulfur bacteria in surface waters at selected stations in the Elliot Lake region.

(a) Denison Uranium Mines at Long Lake

Date	Sample	Description of Station	pH	Count per 100 ml.	
				S oxidizers	Fe oxidize
Oct.'68	D-0	Denison (Long Lake just below tailings discharge from mill)	-	46,000 *	-
	D-1	Long Lake approx. 50 ft. from discharge to Stollery Lake	6.8	21,000 *	-
	D-2	Long Lake above $BaCl_2$ treatment	6.8	9,300 *	< 3
	D-3	Cinder Creek	-	< 3 *	< 3
July '69	D-1	Long Lake 50 ft. from discharge to Stollery Lake	8.3	1,100	not detecte
	D-2	Long Lake above $BaCl_2$ treatment	6.8	240	" "
	D-3	Cinder Creek	-	< 3	" "
	D-4	Stollery Lake (after $BaCl_2$ treatment)	6.3	23	" "
Sept.'69	D-1	Same descriptions as above		1,100	150
	D-2	" " " "		2,100	75
	D-3	" " " "		9	7
	D-4	" " " "		43	9

* Sulphate reducers not detected in either water samples or bottom deposits.

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TABLE I (b) Bacteriological survey at Rio Algom (Nordic)

Date	Sample	Description of Station	pH	Count per 100 ml. S oxidizers	Count per 100 ml. Fe oxidize
Oct.'68	N-0	Nordic (supernatant in tailings area immediately below discharge from mill)	9.8	15 *	< 3
	N-1	Tailings area supernatant above decant	3.4	110,000 *	1,100
	N-2	Stream below culvert from decant	3.5	230,000 *	460
	N-3	Stream above $BaCl_2$ treatment	3.7	40,000 *	2,300
	N-4	Buckles Creek at Hwy. 108 bridge	4.0	24,000 *	46
July '69	N-1	Same Stations as above	4.2	150	not detecte
	N-2	" " " "	4.2	240	" "
	N-3	" " " "	4.5	240	" "
	N-4	" " " "	-	75	" "
Sept.'69	N-0	Same Stations as above (particulate matter found in sample N-0)	8.0	460,000	46,000
	N-1		7.4	93,000	150
	N-3		6.8	24,000	150

TABLE I (c) Stanrock Uranium Mines bacteriological survey

Oct.'68	S-4	Pregnant leach from mine	2.0	430,000	4,600,000
Sept.'69	S-1	Decant at Stanrock	3.8	4,600	21,000
	S-2	Below decant at culvert	3.8	460,000	75,000
	S-4	Pregnant leach solution	2.0	280,000	20,000

* Sulphate reducers not detected.

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TABLE II - Distribution of sulfur and iron oxidizing autotrophs in profiles from tailings waste area at the Nordic site.

Date	Sample	Profile depth (feet)	pH *	Count per gram air dri tailings waste	
				S oxidizers	Fe oxidiz
July '69	1	0	2.7	280,000	not detec
	2	1	3.7	1,500,000	" "
	4	6	5.5	4,600	" "
	5	7	6.1	280	" "
	6	10	6.5	< 25	" "
Sept.'69	Westlake				
	1	0	3.0	1,600,000	81,000
	2	1	3.0	1,300,000	180,000
	3	3 (at water level)	4.0	26,000	10,000

* pH determinations on dried samples.

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RESULTS AND DISCUSSION:

The populations of autotrophic sulfur and iron oxidizing bacteria found at selected stations in surface waters of the Elliot Lake region are described in Table I.

The results of the bacteriological survey for Denison Uranium Mines at Long Lake are shown in Table I (a). Tailings are discharged from the mill into Long Lake. Located at the distal end of Long Lake from the mill is an earth dam where $BaCl_2$ is added to the water flowing into Stollery Lake for radionuclide and some sulfate removal. Cinder Creek also flows separately into Stollery Lake.

The data for October, 1968 indicates that the population of sulfur oxidizers was greatest at 46,000 per 100 ml. just below mill tailings discharge to Long Lake. A lower count of 9,300 was recorded in the sample from Long Lake above the overflow to Stollery Lake. The water samples from Cinder Creek were considered indicative of the natural waters of the region and served as controls, since no discharge of pollutants entered this stream. In October 1968 and July 1969, no sulfur oxidizers were detected (< 3/100 ml.), while in September 1969 only 9/100 ml. were found in Cinder Creek. During July and September of 1969, sulfur-oxidizing thiobacilli were more numerous in Long Lake (1,100, 2,100) then in Stollery Lake (23, 43).

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It is very doubtful whether growth of the thiobacilli was occurr in this lake, since the pH of these water samples was near neutrality, conditions for optimum growth thus being unsatisfactory. However, acidic conditions in the mill tailings or acid leach water before neutralization and discharge may have been favorable for proliferation of these bacteria which might have entered Long Lake with the discharg from the mill. Dilution of the bacteria may possibly explain the successive decrease in population with increasing distance from the point of discharge. These autotrophic bacteria may be acting as a tracer of tailings discharge, carried passively in the neutral water of Long Lake and becoming diluted out measurably by the time they reach Stollery Lake.

Iron oxidizers were not detected in any of the samples taken during July 1969. Failure to detect this group of bacteria was traced to a fault in the preparation of the culture medium.

In October 1968, no iron oxidizers were found in Long Lake and Cinder Creek. Low counts of iron oxidizing autotrophs were recorded in September 1969 in Long Lake (150 and 75/100 ml.), Stollery Lake (9/100 ml.) and Cinder Creek (7/100 ml.). Low counts of these bacteri in surface water are not surprising, since cells of T. ferrooxidans are usually associated with mineral particles where substrate is more abundant.

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Sulphate-reducing bacteria were absent from surface water and bottom samples of Long Lake. Although sulfate concentration and pH were not limiting factors for growth of Desulfovibrio, the redox potential of the surface water was too high and the concentration of organic matter too low for sulfate-reducing activity to occur.

The bacteriological results for the Rio Algom (Nordic) investigation are given in Table I (b). In October 1968, thiobacilli were found in abundance in run-off water from the tailings area and in Buckles Creek, a stream which receives the run-off from the tailings area. At station N-O in the Nordic tailings area just below the neutralized mill discharge, very low numbers of sulfur oxidizers (15/100 ml.) and iron oxidizers (<3) were found. The alkaline pH of the water (9.8) at N-O would be inhibitory or toxic to the autotrophs, possibly explaining the low populations observed. At station N-1, the pH of the surface water in the tailings area above the decant had dropped to 3.4 and the numbers of sulfur and iron oxidizers had increased considerably to 110,000 and 1,100 per 100 ml. respectively. The pH at stations N-2 and N-3 in the effluent stream below the culvert from the tailings area decant, remained acidic at 3.5 and 3.7. At N-2 and N-3, sulfur oxidizers attained populations of 230,000 and 40,000 respectively and iron oxidizers, levels of 460 and 2,300 respectively. Counts at the Buckles Creek station below the discharge from tailings effluent indicated the presence of 24,000 sulfur oxidizers and 46 iron bacteria per 100 ml. of the acidic water.

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In July and September of 1969, the pH of the water samples from Nordic indicated an increase from the previous year. In July, numbers of sulfur oxidizers were lower than those determined in October, ranging from 75/100 ml. in Buckles Creek to 240 in the run-off stream from the tailings area. Populations of iron and sulfur bacteria showed substantial increases in September. Respective counts of sulfur and iron oxidizers were 93,000 and 150 at station N-1 and 24,000 and 150 at N-2. Highest counts for September were obtained from station N-O in the tailings area where populations reached 460,000 and 46,000 for sulfur and iron bacteria respectively. The sample from N-O contained some particulate material. Bacteria associated with mineral particles or entering the area directly in the discharge of tailings or mine leach water may have contributed to this high count observed at N-O.

Sulfate-reducers were also not detected in any surface water samples taken from the Nordic stations. Environmental conditions in these acid waters are not suitable for growth of Desulfovibrio or sulfate-reducing clostridia.

The bacteriological results of the Stanrock investigation are in Table I (c). Water samples from the tailings area decant at Stanrock showed the highest counts of sulfur and especially iron-oxidizing bacteria, and the lowest pH values of all the mill sites investigated. In September 1969, populations of sulfur oxidizers in the run-off (decant) from the tailings area at stations S-1 and S-2 were found to

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be 4,600 and 460,000. Numbers of iron oxidizers at these stations were 21,000 and 75,000 per 100 ml. respectively.

The acidic pregnant leach from the mine before uranium extraction contained high populations of sulfur and iron autotrophs. Samples taken in October 1968 showed counts of 430,000 sulfur and 4,600,000 iron oxidizers per 100 ml. of mine leach solution. Similar samples obtained in September 1969 indicated populations of sulfur and iron bacteria at levels of 280,000 and 20,000 respectively. Since these thiobacilli are active in the natural leaching of uranium ore, the high populations observed in the acid leach solution are quite conceivable.

In accord with the findings of other workers (45, 23), it was observed that sulfur oxidizing bacteria were generally more abundant than iron oxidizers in surface water samples analyzed, the sulfur group being about 10 times (5x to 100x) as abundant as the iron oxidizers in a given sample. Because sulfur is a better energy source than iron, sulfur oxidizers theoretically can produce more biomass than iron bacteria, i.e. there are more calories yielded per mole of sulfur substrate oxidized than an equivalent concentration of iron metabolized.

Beck & Brown (3) discovered that the efficiency of CO_2 fixation and thus biomass increase was greater when sulfur rather than iron was oxidized by cells of T. ferrooxidans.

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Other possible reasons for the observed lower counts of the iron group in the surface waters are:

(1) The iron oxidizers may have been more difficult to cultivate (fastidious) on the medium used than the sulfur oxidizers.

(2) The iron oxidizers may have been more directly adsorbed to or associated with pyrite particles than the thionic bacteria.

It should be emphasized that the significance of the sulfur oxidizers in these waters cannot be overlooked, since these bacteria are capable of lowering the pH by generating sulfuric acid.

Data on the vertical distribution of sulfur and iron-oxidizing autotrophs at various depths in the solid tailings waste at the Nordic site is presented in Table II.

In July 1969, profile samples of solid tailings waste were taken in the tailings area from the surface down to 10 feet. The pH increased from 2.7 at the surface to 6.5 at 10 feet, while the population of sulfur oxidizers decreased from approx. one million near the surface to < 25/g at the 10 feet depth.

In September 1969, both sulfur and iron oxidizing bacteria were discovered in samples from a profile of an old tailings area at West Lake (Nordic). The pH at the surface and at the water level, 3 feet below was recorded as 3.0 and 4.0 respectively. Numbers of sulfur oxidizers declined from approx. 1.4 million per gram near the surface to 26,000/g at 3 feet; the iron bacterial population decreased from approx. 100,000 near the surface to 10,000 at a depth of 3 feet.

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The higher numbers of these bacteria observed at the surface than at lower depths in the profile may be attributed to improved growing conditions for the bacteria near the surface of the tailings impoundment, with a lower pH of 3.0 and more availability of oxygen than the more reducing and neutral environment at the lower depths. In a given environment, high bacterial counts were related to acidity.

From the results of this investigation, it can be concluded that sulfur and iron-oxidizing bacteria of the Thiobacillus - Ferrobacillus group are found in relative abundance in surface waters polluted by uranium tailings wastes and in the solids of the tailings areas. Appreciable populations of these autotrophic bacteria were not found in the natural, unpolluted waters of Elliot Lake region, as evidenced by the lack of these microorganisms in samples from Cinder Creek.

This bacteriological parameter would appear to have some potential as a sensitive indicator in assessing water pollution by discharges of acid mine drainage and tailings wastes.

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II. - SOME ENVIRONMENTAL FACTORS INFLUENCING ACID FORMATION FROM AQUEOUS SUSPENSIONS OF URANIUM TAILINGS WASTES - (BIOLOGIAL and NON-BIOLOGICAL CONTRIBUTIONS TO SULFURIC ACID FORMATION).

It has been definitely established that the biological or non-biological oxidation of pyritic minerals leads to the formation of sulfuric acid (2, 6, 9, 15, 20, 22). It has been discovered that certain microorganisms in nature play an important role in the production of acidity in coal mines and in the oxidation of metallic sulfides (4, 5, 23).

In neutral or alkaline oxygenated waters, a rapid non-biological atmospheric oxidation of ferrous iron takes place. In acid environments below a pH of 4.0 - 4.5, although there is no appreciable chemical oxidation of ferrous salts, the autotrophic sulfur and iron oxidizing bacteria are capable of effecting the oxidation of ferrous iron to the ferric form (2, 18, 20, 23, 44).

Iron commonly occurs in natural ore bodies as pyrite (FeS_2). Complete oxidation of this sulfide mineral yields ferric hydroxide and sulphuric acid. Environmental factors controlling the rate of pyrite oxidation are oxygen concentration or Eh of the system, pH, temperature, particle size and presence of impurities)9).

The optimum temperature for the biological leaching of metallic sulfide was found to be about 35 °C, but slow bacterial leaching was observed at temperatures as low as 3° - 6°C (13). The oxidizing bacteria were inhibited above 40 °C, but an increased rate of pyrite

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oxidation by a non-biological (chemical) mechanism was observed at higher temperatures near 75 °C (6).

The larger surface area provided by the small grain size of pyrite and a higher oxygen content, enhances the rate of pyrite oxidation. Activity of thiobacteria also greatly increases the oxidation rate of sulfur, ferrous iron and pyrite (2).

Calcium carbonate can retard the oxidation of pyrite by raising the pH of the liquid near the reacting surface, thereby facilitating the precipitation of ferric hydroxides which would impede the movement of oxidizing agents to the reacting mineral surface (9).

The possibilities in the oxidation of pyrite by the iron-oxidizing thiobacilli have been discussed by several authors (3, 9, 14, 39). In summary, these are:

(1) oxidation of pyrite by acidic ferric sulfate, with bacteria oxidizing the resultant ferrous iron to regenerate the oxidant, ferric iron.

(2) direct bacterial action on the ferrous iron moiety of the pyrite.

(3) direct bacterial action on the sulfide portion of the mineral.

(4) a combined action involving two or more of these alternatives.

Pyrite is quite insoluble in water, but conducts electricity. Clark (9) discussed an electrochemical mechanism whereby the oxidation of pyrite can be accomplished by the reduction of ferric ions which act as the oxidant in an electrochemical system. He suggested the rate limiting step was probably the initial reaction resulting in iron and sulfur going into solution from pyrite.

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Silverman (39) presented evidence to show that both microbial and chemical oxidation of pyritic minerals can occur simultaneously.

Although dissolved oxygen accelerates the oxidation of pyrite and the formation of acid, the absence of oxygen may not necessarily prevent these reactions from taking place (2). It has been demonstrated that ferric ions even oxidize pyrite in the absence of oxygen and bacteria (2, 6, 9, 39).

It is probable that two mechanisms of bacterial pyrite oxidation operate concurrently: the direct contact mechanism which requires physical contact between bacteria and pyrite particles for biological oxidation of pyrite, and the indirect contact mechanism according to which bacteria oxidize Fe^{+2} ions to the Fe^{+3} state thereby regenerating the Fe^{+3} ions required for the chemical oxidation of pyrite (39).

Ferric iron whether produced biologically or chemically may react directly with pyrite by the reaction:

$$14\ Fe^{+3} + FeS_2 + 8H_2O \longrightarrow 15\ Fe^{+2} + 2SO_4 = + 16\ H^+$$

which lowers the pH to about 2.7. However, large concentrations of ferric iron would be required to oxidize significant quantities of FeS_2 (9).

During pyrite oxidation, iron and sulfide moieties may be attacked simultaneously and independently by bacteria (14). Bryner & Jameson (5) observed no chemical oxidation of pyrite in a nutrient solution when ferrous or ferric ions were not present, but biological oxidation did occur under the same conditions. Duncan et al. (13) also found no chemical oxidation of pyrite when the sulfide mineral

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was added as sole sources of both iron and sulfur to a synthetic medium, 9K. In attacking the sulfide moiety of the pyritic mineral, the bacteria convert the sulfide to sulphuric acid and the metal ions are free to go into solution. Depending on the pH, Fe^{+2} ions are oxidized to Fe^{+3} ions either biologically or chemically. Subsequent hydrolysis of ferric sulfate produces insoluble ferric hydroxide and more H_2SO_4 which further lowers the pH of the environment.

Beck & Brown (3) observed that the addition of Fe^{+3} to pyrite suspensions resulted in a greater rate of H^+ and SO_4^{-2} production which suggested that FeS_2 and Fe^{+3} reacted chemically to generate H^+ and SO_4^{-2} ions.

Tailings wastes from uranium mining and milling operations in the Elliot Lake region contain appreciable quantities of pyrite along with sulfides of some other metals. A series of experiments was conducted in the laboratory to determine the possible role of sulfur and iron oxidizing bacteria in the generation of sulfuric acid from tailings waste, and to study the effects of some environmental conditions on the acid forming process.

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II. (a) - Sulfuric acid formation from tailings waste slurry under sterile and anaerobic conditions.

Procedure:

Air-dried samples of recently processed tailings from a uranium mill were used in the experiments. Six hundred ml. of a 5% (W/V) suspension of the grey powdered tailings in distilled water was placed into each of six 1000 ml. erlenmeyer flasks. Three flasks served as non-sterile controls, and three flasks were sterilized by autoclaving at 121°C for 60 min. Both control and sterile samples were subjected to 3 treatments:

(1) no treatment

(2) flasks inoculated with culture of *Thiobacillus ferrooxidans* *

(3) flasks inoculated with culture of *T. thiooxidans* *.

After treatments, the pH values of all flasks were initially adjusted to 7.5 with sterile IN NaOH solution. All flasks were incubated at room temperature (approx. 25°C) in the dark; pH determinations were made on the flask contents at least once a week.

Another set of 6 flasks was set up as described previously, except that additional purified pyrite (FeS_2) was added at a concentration of 0.5% to each flask.

* Cultures of iron oxidizers (*T. ferrooxidans*) and sulfur oxidizers (*T. thiooxidans*) were isolated from a mine tailings area and grown on ferrooxidans and thiooxidans broths respectively.

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An experiment to determine the effect of two sterilization methods and anaerobiosis on the rate of acid production from tailings suspensions was also carried out. Tailings suspensions (5%) were prepared in screw-capped flasks and subjected to the following treatments:

(1) sterilized by autoclaving - 15 p.s.i./60 min. - aerobic incubation

(2) sterilized by gamma-irradiation - dose level of 5 megarads in a Gamma cell 220 (Co^{60} source) - aerobic incubation

(3) non-sterile control - incubated aerobically

(4) non-sterile control - incubated anaerobically in a Brewer anaerobic jar with a "Gas-pak" system.

The pH of all samples was initially adjusted to 8.0 after sterilization. All samples were incubated at approx. 25 °C in the dark and the pH's were determined periodically.

Results and Discussion:

The pH changes occurring in the sterilized tailings slurry which were incubated with the bacterial cultures are depicted in Fig. 1. The pH dropped rapidly at a constant rate from 8.0 to 6.0 during the first week in both the sterile control and the sterilized samples inoculated with the cultures of sulfur and iron oxidizing bacteria, T. thiooxidans and T. ferrooxidans. After the first week, the rate of acid production of the inoculated treatments exceeded that of the sterile control. At 6 weeks, the pH of the control

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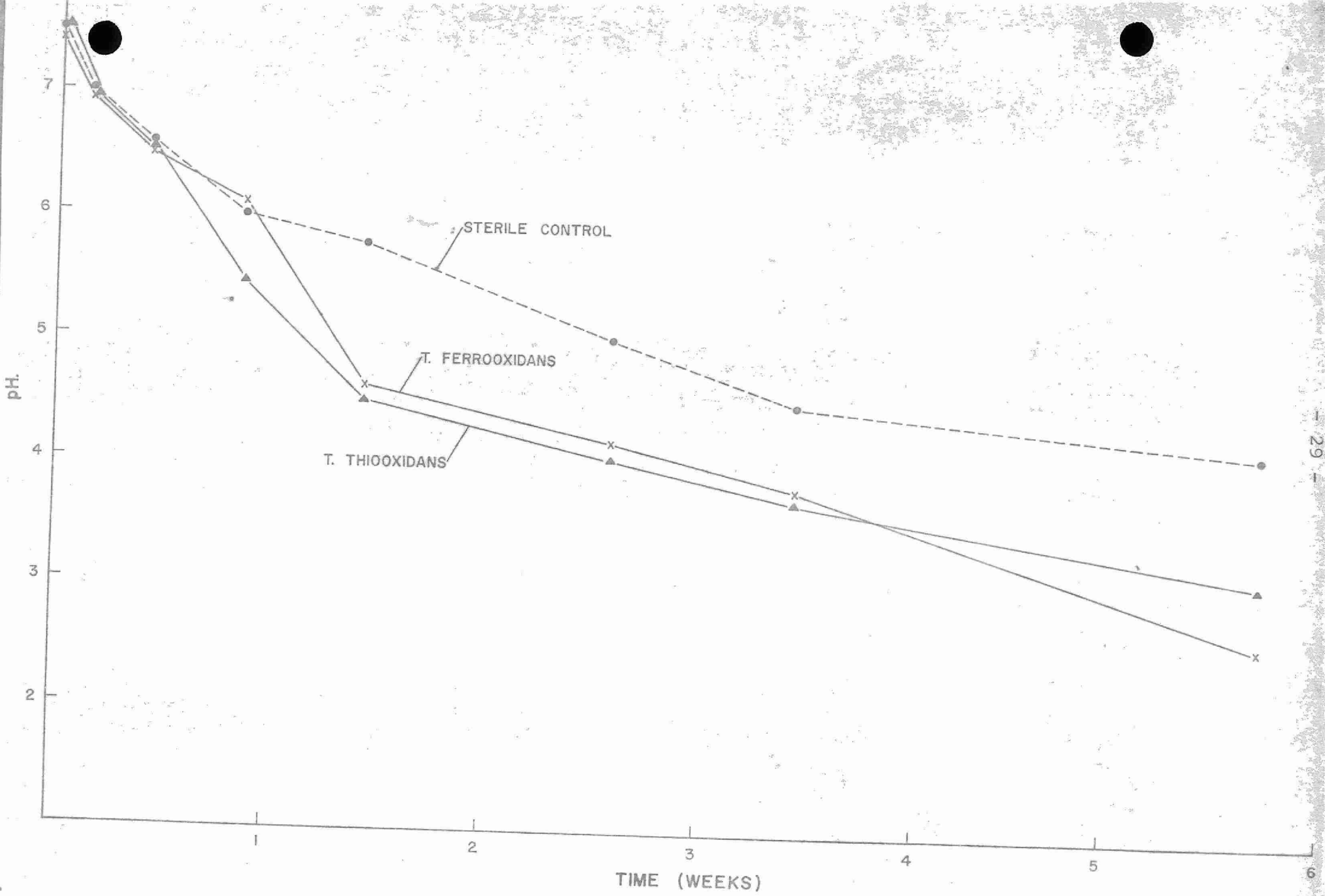

FIG. 1 pH CHANGES IN URANIUM TAILINGS SLURRY OCCURING AFTER AUTOCLAVING AND INOCULATION WITH CULTURES OF SULFUR AND IRON BACTERIA

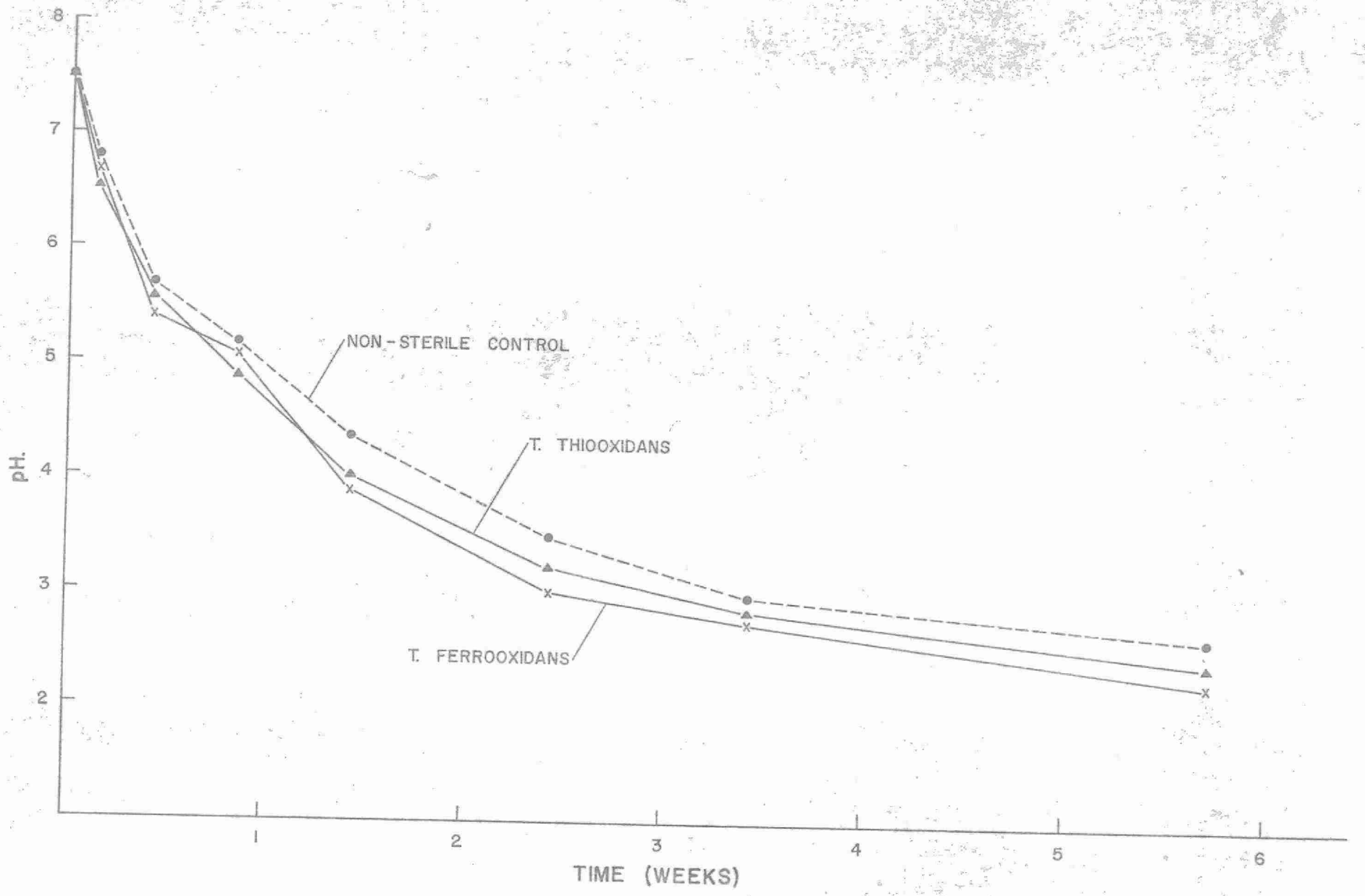

FIG. 2 pH CHANGES IN URANIUM TAILINGS SLURRY OCCURING IN NON-AUTOCLAVED SAMPLES AFTER INOCULATION WITH CULTURES OF SULFUR AND IRON BACTERIA.

reached 4.2 compared to values of 2.7 and 3.2 for the inoculated samples. By 16 weeks, the rate of acid formation had levelled off in all samples, with pH values of 3.5, 1.7 and 2.1 recorded for the sterile control and flasks inoculated with iron and sulfur oxidizers respectively. At this time the color of the tailings had changed from the original grey to a reddish-brown to yellow color in the flask inoculated with the T. ferrooxidans culture. The tailings color remained grey in the sterile control and the flask inoculated with the T. thiooxidans culture.

Fig. 2 illustrates the pH changes occurring in the non-sterile uranium tailings suspensions. No significant difference was observed in the rate of acid production between the non-sterile control and the non-sterile but inoculated treatments. The rate of acid production was greatest during the first week, the pH of the samples having dropped from 8.0 to approx, 5. The rate declined gradually after 1 week with pH values recorded at 6 weeks of 2.5, 2.3 and 2.4 for the non-sterile samples. At 16 weeks, the pH of all three samples had reached about 1.8, and the color of the tailings in all flasks had changed to the yellow-reddish-brown, an indication of oxidized iron. The addition of extra pyrite to a series of flasks treated in the same way had no effect on the rate of acid production. It is thus apparent that pyrite is not a limiting substrate in the tailings waste.

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Fig. 3 indicates the rates of sulfuric acid production in tailings wastes subjected to aerobic and anaerobic conditions and to two methods of sterilization. The pH declined without a lag in all the samples except the non-sterile slurry incubated anaerobically. In this case, the pH remained near 8.0 for the first three weeks. The after the rate of acid production began to decline and levelled off at 5 weeks incubation. The pH remained relatively constant at 6.2 in the interval from 5 to 16 weeks. The most rapid rate of acid formation was observed in the non-sterile control flask incubated aerobically. The pH dropped most rapidly during the first month and subsequently levelled off to reach 1.8 at 16 weeks.

The rate of acid formation in the tailings sterilized by gamma-irradiation and autoclaving followed similar trends as the non-sterile control, but the pH values recorded for the sterile samples were not as low as those for the control. More acidity was generated in the autoclaved tailings than in the gamma sterilized sample. At the end of the experimental period, the pH values for the gamma-sterilized and autoclaved samples were recorded as 3.4 and 2.7 respectively.

The increased quantities of acid produced or lower pH values obtained in the non-sterile tailings suspensions as compared to the sterile samples can be attributed to bacterial activity in the former. The pH eventually levelled off and remained near 3.0 in the sterile samples while in the samples containing the thiobacteria, the pH dropped to as low as 1.7.

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Other workers (4, 38) have observed that sulfide ore inoculated with sulfide-oxidizing bactera was solubilized to a greater extent and gave more acid production than sterilized ore not inoculated with bacteria. Razzell and Trussell (35) showed that bacterial leaching was more effective in the solubilization of pyrite than non-biological leaching with ferric sulfate.

Since the pH of the sterile tailings samples did fall from the initial value of 7.5 - 8.0, it was apparent that a chemical process was playing a role in the production of acidity from tailings slurries. When the pH of the sterile tailings suspension was initially brought to 8.0, air oxidation of ferrous iron in solution could readily occur. The resultant ferric ions would then be able to react directly with the pyrite particles to regenerate more ferrous iron and sulphuric acid which would depress the pH. When the pH of the sterile tailings decreased to about 4.0, chemical oxidation of ferrous iron would be prevented, and in the absence of iron-oxidizing bacteria, ferric iron would eventually become depleted in the system and the electrochemical process would come to a halt, hence the stabilization of the pH in the sterile system above 3.0. In the presence of iron-oxidizing bacteria (non-sterile samples), biological oxidation of ferrous iron at pH values less than 4.0 would occur and additional oxidant (Fe^{+3}) would be produced to react with pyrite and depress the pH below 2.0.

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It can be concluded also that the sulfur and iron oxidizing bacteria were present originally in the tailings samples since no differences in the rates of acid formation were observed in the non-sterile tailings suspensions whether inoculated or not with cultures of sulfur and iron bacteria (Fig.2). Significantly more acidity was produced in sterile tailings inoculated with bacterial cultures than in the sterile control (Fig. 1). This difference in acidity could be attributed to microbiological activity on pyrite in the tailings.

Beck & Brown (3) observed that all their isolates of thiobacteria that were able to use sulfur were also able to use sulfide ores as energy sources. Apparently the sulfur and iron oxidation systems of T. ferrooxidans are different and the former is essential for the solubilization of sulfide ores.

The sulfur bacteria which were isolated on thiooxidans broth and inoculated into the sterile tailings apparently cause the production of H_2SO_4 from the oxidation of pyrite. The only tailings samples not showing evidence of iron oxidation after prolonged incubation were the sterile control and the sterile sample inoculated with the T. thiooxidans culture. This culture apparently caused oxidation of pyrite sulfur since more H_2SO_4 was produced in the inoculated treatment than in the sterile control but the bacteria did not oxidize iron. The culture of iron bacteria (T. ferrooxidans) inoculated into the steril tailings generated H_2SO_4 but also oxidized iron.

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These results would suggest that the sulfur oxidizers (Thiobacillus thiooxidans) produced sulfuric acid from the biological oxidation of the sulfide moiety of pyrite with the release of the pyrite iron as Fe^{+2} which was not oxidized in acid solution, since below a pH of 5.0, the rate of chemical oxidation of Fe^{+2} by oxygen is slow. T. ferrooxidans apparently oxidized both ferrous iron to the ferric form and oxidized pyrite sulfur to H_2SO_4, although some of the H_2SO_4 produced in this case may have resulted from the strictly chemical reaction of ferric iron (generated biologically under acid conditions) with pyrite.

The pH of the tailings sample incubated anaerobically did not fall below 6.2 from an original value of 8.0, and no evidence of iron oxidation was observed. Ferric ions in the suspension originally may have reacted chemically with pyrite to produce sulfuric acid and ferrous ions. However, under the anaerobic conditions imposed upon this system, no chemical or biological oxidation of iron could possibly occur. With no regeneration of Fe^{+3} ions at the low Eh of the anaerobic system, the original supply of Fe^{+3} would become depleted after reaction with pyrite and the production of sulfuric acid from pyrite oxidation would be halted.

Although chemical processes are involved in the oxidation of the tailings pyrite, it also appears that both the sulfur and iron oxidizing autotrophic bacteria, Thiobacillus thiooxidans and

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T. ferrooxidans play a significant role in the oxidation of pyrite with the production of acidity from tailings suspensions. The iron oxidizing bacterium, T. ferrooxidans is responsible for the oxidation of ferrous iron in the acidic tailings suspensions.

The acid forming process in the tailings suspension most likely involves the bacterial oxidation of Fe^{+2} to Fe^{+3}, the oxidant which reacts chemically with pyrite to directly form more Fe^{+2} and H_2SO_4. Acidity is also produced by dried attack of the pyrite sulfur by the sulfur oxidizers to form H_2SO_4, while Fe^{+2} ions are free to go into solution.

As long as Fe^{+3} ions are present in the system, the oxidation of pyrite with generation of H_2SO_4 can occur by an electrochemical process.

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II (b) - Effect of inorganic phosphorus compounds and EDTA on acid production in suspensions of uranium mill tailings.

With the premise that ferric ions in tailings suspensions catalyze the oxidation of pyrite, an experiment was conducted to determine if chemicals which are capable of removing ferrous and ferric ions from solution could prevent the formation of sulphuric acid from tailings pyrite.

Procedure:

A series of 1 liter erlenmeyer flasks containing a 5% suspension of tailings was subjected to various treatments:

Flasks	Treatment
1	Control - nothing added
2	orthophosphate (3000 ppm K_2HPO_4)
3	" (1000 ppm ")
4	" (100 ppm ")
5	pyrophosphate (1000 ppm sodium salt)
6	" (100 ppm " ")
7	Calgon (1000 ppm sodium hexametaphosphate)
8	" (100 ppm " " ")
9	EDTA (1000 ppm ethylenediamine-tetracetic acid as the disodium magnesium salt)
10	EDTA (100 ppm).

After the added chemicals were mixed with the tailings suspensions, the pH of all flask contents was adjusted initially to 8.0. Flasks were incubated at room temperature (approx. 25 °C) and checks were kept on pH of flask contents.

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Results and Discussion:

The production of acidity in uranium tailings suspensions receiving the various chemical treatments is illustrated graphically in Fig. 4. The pH of the tailings receiving no treatment (control), and EDTA (100 ppm) decreased rapidly over the initial 4 week period, falling from 8.0 to approx. 2.5 at 4 weeks. Thereafter the rate of acid production levelled off, the pH values after 24 weeks reaching 2.0 for the control and 2.1 for the EDTA treatment.

The rate of acid formation in the tailings suspensions containing orthophosphate, pyrophosphate and hexametaphosphate at 100 ppm was greatest during the first two weeks approaching the control rate and thereafter declining. At 8 weeks, the pH of these samples had reached about 2.3. At 24 weeks incubation, iron oxidation was evident in the tailings samples treated with EDTA, the 3 phosphate compounds at 100 ppm and the control.

The chemical additives at the higher concentrations significantly inhibited the depression of pH in the tailings. In tailings samples treated with EDTA, orthophosphate and pyrophosphate all at 1000 ppm, the pH was found to decline sharply for the first two weeks at a rate approaching that of the control, reaching values near pH 6.0 at 2 weeks. However, after 2 weeks, the rates of acid production drastically declined in these samples, reaching pH values near 5.0 at 8 weeks. By 24 weeks, the pH in the EDTA (1000 ppm) - treated sample had fallen to 2.9 and the flask contents showed visual evidence of iron oxidation.

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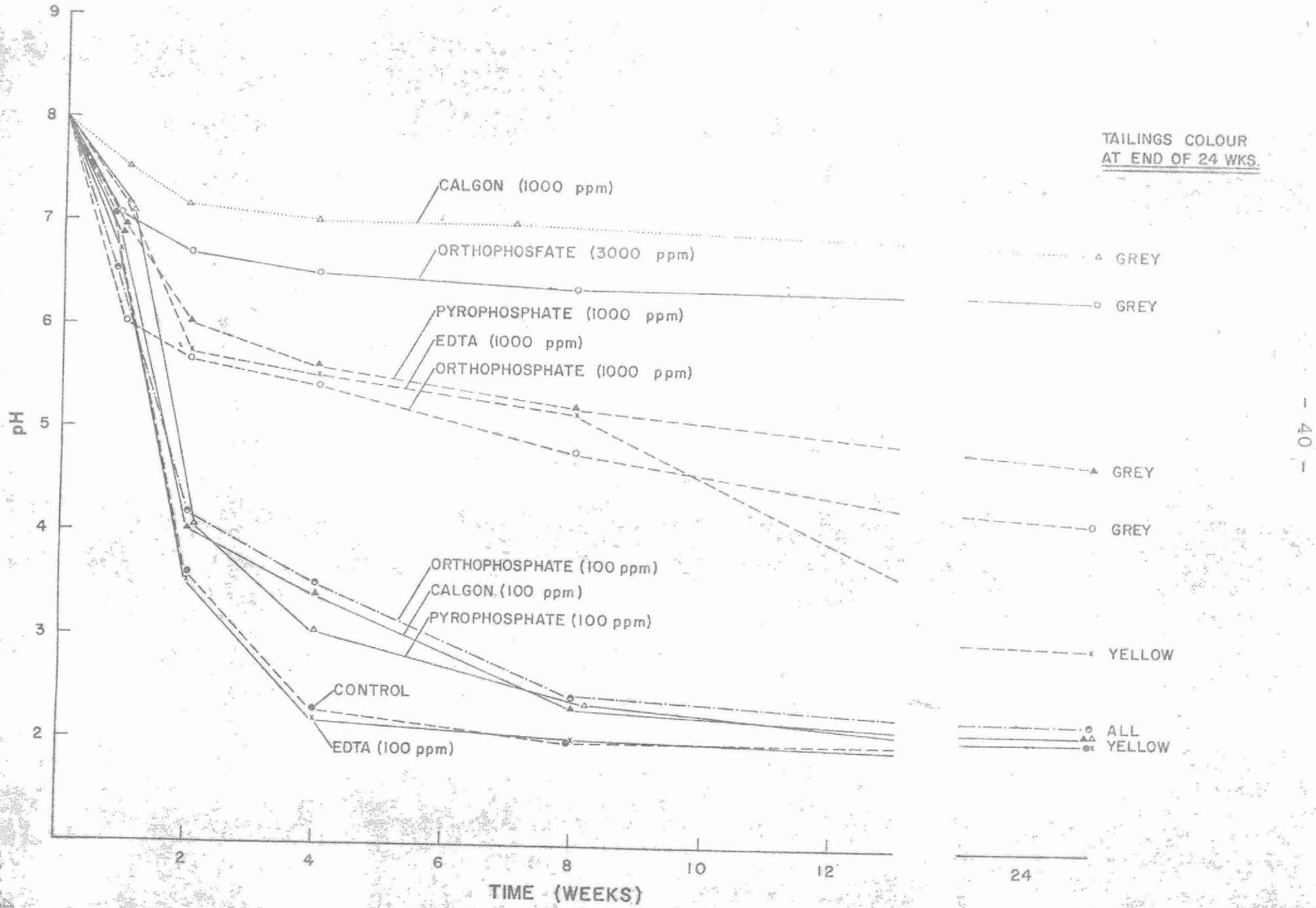

FIG. 4 EFFECT OF VARIOUS CHEMICAL

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In contrast to EDTA, the pyrophosphate and orthophosphate at 1000 ppm maintained the pH relatively well, the pH values for pyrophosphate and orthophosphate treatments at 24 weeks being recorded as 4.4 and 4.0. There was no evidence of iron oxidation of the tailings treated with these phosphates at 1000 ppm.

Orthophosphate at 3000 ppm and calgon at 1000 ppm stabilized the pH, the greatest pH decline occurring during the initial two weeks. By 24 weeks, the pH of the tailings samples treated with orthophosphate (3000 ppm) and calgon (1000 ppm) had reached 6.2 and 6.5 respectively with no oxidation of iron apparent in the samples.

All the chemicals added at 100 ppm concentration to the tailings had no significant effect in stabilizing the pH or preventing iron oxidation. At 1000 ppm, pyrophosphate and orthophosphate were more effective in maintaining the pH for a long period than was EDTA. Calgon was the most effective agent in stabilizing the pH of the tailings suspension.

Razzell and Trussell (35) decreased the rate of pyrite oxidation by changing the concentration of KH_2PO_4 above or below 0.1%. High phosphate concentrations either inhibited the release or precipitated th Cu^{+2} and Fe^{+3} ions as insoluble phosphates. Alkaline pH's favour the surface precipitation of ferric hydroxide or basic ferric sulfate which presents a physical barrier to microbial or chemical attack of the surface of pyrite particles.

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Silverman and Lundgren (40) observed that the growth of F. ferrooxidans was completely inhibited by a K_2HPO_4 concentration of 2.75 g/l or more.

Naturally occurring chelators, humic acids may complex with ferrous or ferric ions in water. Because such organo-metallic complexes oxidize slowly, they are sometimes responsible for the slow rates of ferrous iron oxidation and deposition of ferric hydroxide in acid drainage (2).

Calgon (hexametaphosphate) and EDTA are chelating or sequestering agents, and may form complexes with metallic cations in solution, e.g. Fe^{+2} thereby protecting the metal from oxidation. Orthophosphate and pyrophosphate, on the other hand remove iron from solution by reaction with Fe^{+3} to form insoluble precipitates of ferric phosphates.

Concentrations of the chemicals at 100 ppm were not enough to remove the excess iron from solution. Concentrations of phosphate compounds near 1000 ppm were required to stabilize the pH of the 5% tailings suspensions.

In the case of chelation by calgon and EDTA or precipitation by orthophosphate and pyrophosphate, Fe^{+2} or Fe^{+3} ions would be removed from solution and made unavailable for further reaction and oxidation of pyrite in the tailings, thereby preventing formation of sulfuric acid.

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In the case of EDTA treated tailings, the pH had dropped and some iron had become oxidized by 24 weeks. The organic moiety of the EDTA-iron complex may have been degraded, rendering Fe^{+2} available for oxidation and reaction with pyrite to form H_2SO_4.

The mechanism involved in the stabilization of pH is more likely by iron removal than by a buffering capacity of phosphates. If buffering were the only cause, when the reaction of H^+ ions (which would be continuously generated) with the basic phosphate went to completion, the pH would be expected to drop markedly. However, this was not the case observed with the polyphosphates.

II. (c) - Effect of Calgon on growth of acidophilic thiobacteria in uranium tailings suspensions.

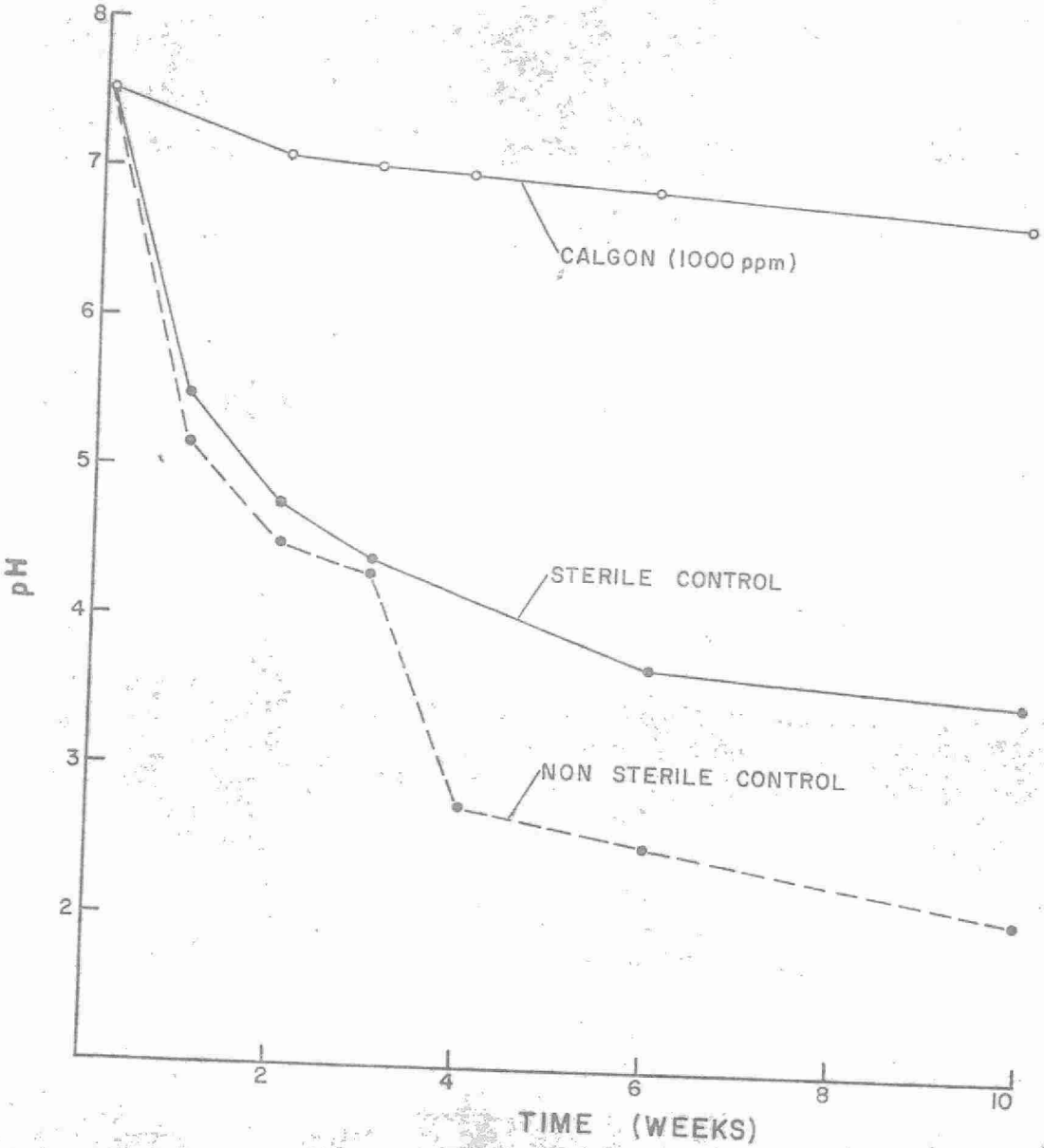
Procedure:

To determine if Calgon had any effect on the growth of acidophilic thiobacilli in tailings suspensions, flasks containing 5% tailings were prepared as previously described and treated as follows: One flask was sterilized by autoclaving, a second was held as a non-sterile control and a third was treated with calgon (sodium hexametaphosphate - 1000 ppm). The initial pH of all samples was brought to 7.5 with sterile IN NaOH solution; pH's were recorded and MPN counts were carried out on all samples for sulfur-oxidizing thiobacteria using thiooxidans broth as described in Methods of Part I.

Results and Discussion:

The changes observed in populations of autotrophic sulfur oxidizers and the pH values in uranium tailings slurries receiving the described treatments are shown in Fig. 5.

The pH of the tailings suspension treated with calgon did not fall below 6.6 in the 10 week period studied. The pH of both the sterile and non-sterile tailings samples decreased rapidly at about the same rate from 7.5 initially to 4.4 at 3 weeks. At 4 weeks, however, the pH of the non-sterile control tailings had decreased to 2.8 compared to a value of 3.9 in the sterile control. At 10 weeks, the pH of the non-sterile and sterile tailings had reached 2.1 and 3.5 respectively.



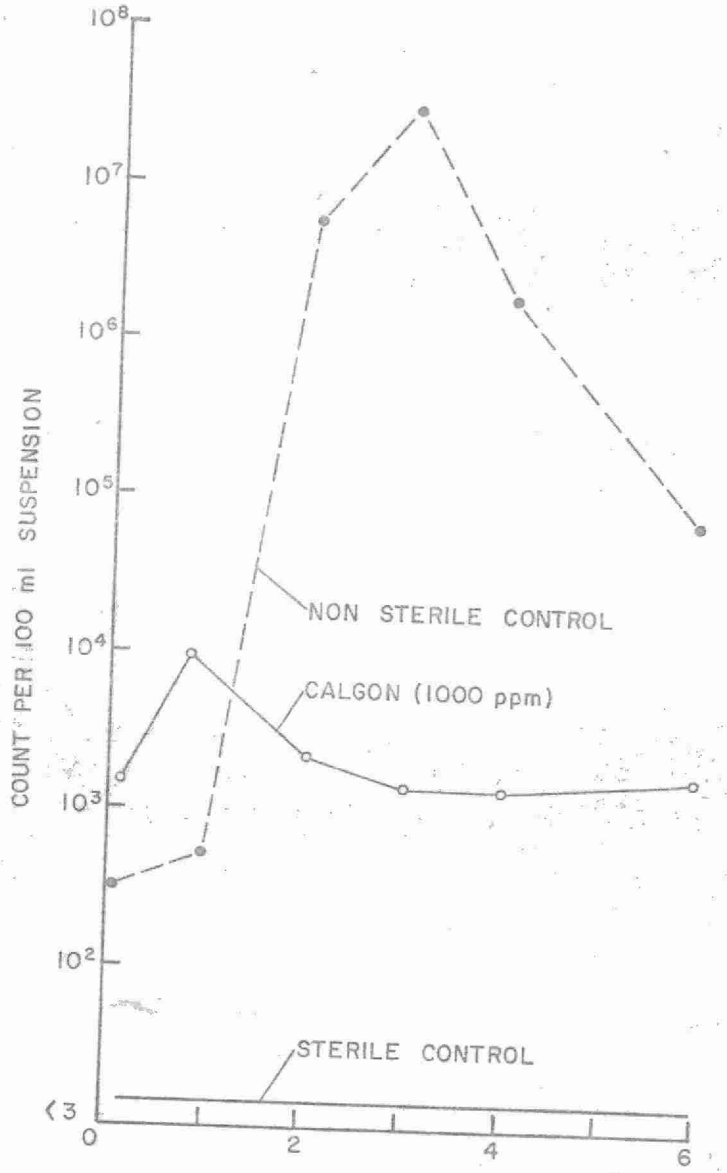


FIG. 5 EFFECT OF CALGON ON GROWTH OF T. THIOOXIDANS IN URANIUM TAILINGS SUSPENSION

The populations of acidophilic Thiobacillus sp. in the tailings samples treated with Calgon did not increase significantly over the 6 week period, the population remaining static at about 2.0×10^3 bacteria per 100 ml. suspension.

In the non-sterile sample, no multiplication of the bacteria occurred during the first week. At 2 weeks, the population of sulfur bacteria reached 5.0×10^6/100 ml. and at 3 weeks attained a maximum cell concentration of 6.0×10^7/100 ml.

The population of sulfur oxidizers showed a subsequent decline reaching 3.0×10^6 and 6.0×10^4 at 4 and 6 weeks respectively.

No oxidizing bacteria were detected at any time in the autoclaved tailings sample, indicating that sterile conditions were maintained during the experimental period. (All counts expressed as <3/100 ml.) The pH depression in the sterile control could therefore be attributed to a non-biological process.

In the calgon-treated sample, no growth of the bacteria occurred because the pH of the environment was above the maximum limit for growth of these acidophilic microorganisms.

In the absence of calgon, chemical oxidation of pyrite can depress the pH of the tailings low enough for growth of the thiobacilli to commence. As the bacteria multiply and metabolize pyrite, more H_2SO_4 is formed and the pH is lowered further. It was observed that acid production showed a marked increase between 3 and 4 weeks incubation when the population of sulfur bacteria reached maximum numbers.

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It is thus apparent that the autotrophic sulfur oxidizing bacteri are capable of metabolizing the sulfur from tailings pyrite in acid environments with the resultant production of sulfuric acid from this biological process.

Summary

1. In the Elliot Lake area, autotrophic sulfur and iron oxidizing thiobacilli were found in significantly greater numbers in surface waters polluted by uranium tailings wastes than in uncontaminated water. Highest populations of these bacteria occurred in the tailings areas and were usually associated with acidic conditions.
2. The formation of sulfuric acid from the oxidation of pyrite in tailings suspensions involved two processes. In a neutral or slightly alkaline milieu, chemical oxidation depressed the pH to about 3.0. In acidic environments, microbiological activity lowered the pH to 1.5. Both sulfur and iron oxidizing bacteria were involved.
3. The acid forming process from tailings suspensions in a closed system is prevented by anaerobic conditions.
4. Chemicals, e.g. inorganic phosphates, EDTA which remove soluble iron from solution by chelation or precipitation, inhibited pyrite oxidation in 5% tailings suspensions at concentrations of 1000 ppm but not at 100 ppm.

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Acknowledgments

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